

helices is significantly different with bulky hydrophobic residues buried deep in the membrane. Salient features of the structure will be reported in light of proton transport mechanism.

253-Pos

Site-Directed Spin-Label EPR Studies Report on Drug-Induced Conformational Change of Influenza A M2 Protein

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The M2 protein from influenza A is a pH-activated proton channel that plays an essential role in the viral life cycle and serves as a drug target. Using spin labeling EPR spectroscopy we studied a 38-residue M2 peptide spanning the transmembrane region and its C-terminal extension. We have obtained residue-specific environmental parameters in the presence of the antiviral drug amantadine to gain information about the drug bound state of M2 in POPC/POPG lipid bilayers. Power saturation studies of spin-labeled peptides reconstituted in a DOGS-NTA(Ni)-containing bilayers report on the accessibility of spin labels to nickel(II) chelated at the aqueous-lipid interface.

254-Pos

HIV-1 Matrix Binding to Model Membranes Investigated By Neutron Reflectivity: Electrostatics and Binding Orientation

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The N-terminal Matrix (MA) domain of the HIV-1 Gag protein is responsible for binding the membrane during viral assembly. A basic patch of residues localized in the MA domain confers a strong electrostatic component to this binding interaction. Through mutagenesis the putative binding interface of MA has been mapped out, but not directly determined by experimental measurements. We present neutron reflectivity measurements that resolve the one dimensional scattering length density profile of MA bound to a lipid membrane. The model membrane system used maintained the anionic surface charge density of the native viral membrane. Molecular refinement using atomic structures of MA suggests an orientation of the protein on the membrane consistent with previous mutagenesis and electrostatic modeling studies. Remarkably the MA protein maintains this orientation without the presence of the post-translational myristate group. Furthermore MA is found to only peripherally penetrate the membrane headgroups by 4.8 ± 1.2 Å allowing only amino acid side chains to contact the lipid headgroups. Our results find that electrostatic interactions are sufficient to preserve the correct binding motif of MA with the viral membrane.

255-Pos

Oligomerization of Transmembrane Alpha-Helices Modulated By C-terminal Boundary Residues

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In studies of the structural biology of membrane proteins, the success of strategies based on the "divide-and-conquer" approach, where peptides are used to model the individual transmembrane (TM) α -helices of membrane proteins, depends upon the correct identification of the membrane-embedded TM α -helix amino acid sequence within the full-length protein. In the present work, we examine the effects of excluding or including TM boundary residues on the intrinsic properties of the TM2 α -helix of myelin proteolipid protein (PLP). Using protein gel electrophoresis, circular dichroism, and fluorescence resonance energy transfer in the membrane-mimetic detergent sodium dodecylsulfate (SDS) to study parent sequence KKKK-⁶¹AFQYVIYGTASFFFLYGALL-LAEG⁸⁹-KKKK - along with analogs containing an additional wild type Phe-90, Phe-90 and Tyr-91, and a hydrophobic mutant Leu-90 - we demonstrate that the removal of a single amino acid from the C-terminus of this TM segment is sufficient to change its intrinsic properties, with TM2 61-89 displaying only a monomeric form, but with principally dimers arising for the other three peptides. The findings suggest that deletion of critical C-terminal residue(s) tends to re-position the helix terminus toward the membrane-aqueous interface, and emphasize the potential influence of boundary residues on TM properties when utilizing peptides as models for TM α -helices. These finding may implicate a role for such residues in membrane protein folding and assembly.

256-Pos

Structure, Dynamics and Topology of the N-terminus and First Transmembrane Segment of APJ

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APJ is a G-protein coupled receptor expressed in the cardiovascular system, central nervous system and several other tissues. Activation of APJ by the peptide ligand apelin has defined roles in cardiovascular regulation, in glucose

metabolism and in tumour growth. Transmembrane proteins such as APJ are difficult to study due to expression, solubility and refolding difficulties. For this reason we have produced a fragment of APJ containing the functionally essential N-terminal region and first transmembrane helix of the receptor (APJ55). Through a combination of circular dichroism (CD) and nuclear magnetic resonance (NMR) spectroscopy we have characterized APJ55. CD spectroscopy indicates that APJ55 only properly refolds in specific detergents, with the anionic detergents sodium dodecylsulphate and 1-palmitoyl-2-hydroxy-sn-glycero-3-[phospho-rac-(1-glycerol)] (LPPG) being the best. NMR spectroscopy has provided an initial structure of APJ55. As a complement to this structure, relaxation studies and paramagnetic spin label titration demonstrated the dynamics and topology of APJ55 in the LPPG micelle. Finally the structure of APJ55 has been placed into the context of full length APJ using a homology model. APJ55 provides a new system to probe apelin-APJ interactions and is a basis for study of additional regions of APJ.

257-Pos

CD and EPR Structural Studies on the KCNE1 Protein in a Lipid Bilayer

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KCNE1, also known as minK, is a membrane protein responsible for modulating the KCNQ1 voltage-gated potassium ion channel in the human heart. Previous *in vivo* electrophysiological studies have shown that KCNQ1 loses its functionality in the absence of KCNE1, showing that KCNE1 is an essential protein for proper heart function (Sanders et al., *Biochemistry* 2007 46:11459-11472). Though KCNE1 has been extensively studied in micelle detergent systems, little work has been done to study the protein in an actual lipid bilayer-membrane system. Our current research uses biophysical techniques such as circular dichroism (CD) spectroscopy and electron paramagnetic resonance (EPR) spectroscopy to characterize and compare KCNE1 proteins in various micelle and lipid bilayer environments using both qualitative and quantitative methods. Our CD spectroscopy experiments have shown that KCNE1 undergoes a change in secondary structure when removed from a micelle environment and placed in a lipid bilayer. We have used EPR spectroscopy to show that the dynamic properties of KCNE1 also change when taken out of micelles and inserted into lipid bilayers. Calculations have been done to quantify these differences in the structural and dynamic properties observed for KCNE1 in micelles and lipid bilayers.

258-Pos

Accessory Alpha-Helix of Complexin I Can Displace VAMP2 Locally in the Complexin-Snare Quaternary Complex

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The calcium-triggered neurotransmitter release requires three SNARE (soluble N-ethylmaleimide-sensitive factor attachment protein receptor) proteins: synaptobrevin 2 (or VAMP2) on the synaptic vesicle and syntaxin 1 and SNAP-25 at the presynaptic plasma membrane. This minimal fusion machinery is believed to drive fusion of the vesicle to the presynaptic membrane. Complexin, also known as synaphin, is a neuronal cytosolic protein that acts as a major regulator of synaptic vesicle exocytosis. Stimulatory and inhibitory effects of complexin have both been reported, suggesting the duality of its function. To shed light on the molecular basis of the complexin's dual function, we have performed an EPR investigation of the complexin-SNARE quaternary complex. We found that the accessory α -helix (amino acids 27-48) by itself has the capacity to replace the C-terminus of the SNARE motif of VAMP2 in the four-helix bundle and makes the SNARE complex weaker when the N-terminal region of complexin I (amino acids 1-26) is removed. However, the accessory α -helix remains detached from the SNARE core when the N-terminal region of complexin I is present. Thus, our data show the possibility that the balance between the activities of the accessory α -helix and the N-terminal domain might determine the final outcome of the complexin function, either stimulatory or inhibitory.

259-Pos

Osmolytes Modulate Conformational Transitions in Solvent-Exposed Regions of Two Outer Membrane Proteins

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Electron paramagnetic resonance (EPR) spectroscopic studies using site-directed spin labeling (SDSL) have been used to investigate local structure and conformational exchange in different regions of two *E. coli* outer-membrane TonB-dependent transporters: BtuB and FecA. It is known that the